Short title: Capitulum patterning in *Gerbera hybrida*

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Evolutionary co-option of floral meristem identity genes for patterning of the flower-like Asteraceae inflorescence

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One sentence summary: Highly conserved genes that regulate identity of single flowers in conventional plant models, regulate the unique inflorescence architecture of the evolutionary successful Asteraceae plant family

Footnotes:

List of author contributions: Y.Z., T.Z., S.K.B., S.T., V.A.A., T.H.T. and P.E. conceived and planned the research. S.K.B., T.T. and P.E. supervised the experiments. Y.Z., T.Z., S.K.B., S.T., K.M., T.H.T. and P.E. performed experiments. Y.Z., T.Z. analyzed the data. Y.Z., T.Z. and P.E. wrote the article with contributions of all the authors who reviewed and approved the final article.

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ABSTRACT

The evolutionary success of Asteraceae, the largest family of flowering plants, has been attributed to the unique inflorescence architecture of the family, which superficially resembles an individual flower. Here we show that Asteraceae inflorescences (flower heads, or capitula) resemble solitary flowers not only morphologically but also at the molecular level. By conducting functional analyses for orthologs of the flower meristem identity (FMI) genes *LEAFY* (*LFY*) and *UNUSUAL FLORAL ORGANS* (*UFO*) in *Gerbera hybrida* (*Gh*), we show that *GhUFO* is the master regulator of FMI, while *GhLFY* has evolved a novel, homeotic function during the evolution of head-like inflorescences. Resembling *LFY* expression in a single flower meristem, uniform expression of *GhLFY* in the inflorescence meristem defines the capitulum as a determinate structure that can assume floral fate upon ectopic *GhUFO* expression. We also show that *GhLFY* uniquely regulates the ontogeny of outer, expanded ray flowers but not inner, compact disc flowers, indicating that distinction of different flower types in Asteraceae is connected with their independent evolutionary origins from separate branching systems.

INTRODUCTION

In flowering plants, inflorescences are the branched structures that bear flowers. Their architecture in terms of number and arrangement of flowers shows enormous variation in nature and plays a central role in angiosperm reproductive adaptation and success. Most of our knowledge on the molecular regulation of inflorescence architecture is based on studies of three major inflorescence types: racemes in *Arabidopsis* or snapdragon, cymes in Solanaceae species such as petunia or tomato, and panicles in grasses (Prusinkiewicz et al., 2007; Park et al., 2012; Teo et al., 2014). In the model plant *Arabidopsis*, endogenous and exogenous flowering inducing signals convert the vegetative shoot meristem into an inflorescence meristem (IM) that initiates determinate flower meristems (FMs) on its flanks. The inflorescence forms a simple, indeterminate (monopodial) raceme that elongates and never forms a terminal flower due to maintenance of the stem cells in the central zone of the meristem. In Solanaceae, the cymous IM always terminates in a flower, but forms new axillary IMs that continue growth, leading to a zig-zag-like sympodial branching pattern. Panicles in grasses show more complex lateral branching and both apical and lateral meristems may form flowers. Using mathematical modelling, Prusinkiewicz et al. (2007) showed that a single developmental model (so called 'transient model') can generate the distinct inflorescence types (racemes, cymes, panicles) found in nature.

In all basic inflorescence types, flower meristem identity is controlled by homologs of at least three functionally conserved proteins – LEAFY (LFY), UNUSUAL FLORAL ORGANS (UFO) and SEPALLATA3 (SEP3) – that diverge in their spatiotemporal expression domains leading to differences in inflorescence meristem patterning (Weigel et al., 1992; Souer et al., 2008; Rebocho et al., 2008; Lippman et al., 2008). In *Arabidopsis*, *LFY* is uniformly expressed in floral buds where it specifies FM identity (Weigel et al., 1992). By interacting with the key co-regulators UFO (Chae et al., 2008) and SEP3 (Liu et al., 2009), LFY initiates the floral program by activating flower organ identity genes. Constitutive expression of *LFY* is sufficient to convert both apical and lateral

meristems into terminal flowers (Weigel and Nilsson, 1995) while loss of function alleles result in partial loss of FM identity, converting flowers into shoots (Weigel et al., 1992). Studies in petunia and tomato indicate that functionally similar proteins regulate patterning in cymose inflorescences, however, in an opposite manner compared to *Arabidopsis* (Lippman et al., 2000; Park et al., 2012, Rebocho et al., 2008; Souer et al., 2008). For example, the *LFY* homologs *ABERRANT LEAF AND FLOWER* (*ALF*) in petunia and *FALSIFLORA* (*FA*) in tomato show more ubiquitous expression during vegetative growth (Souer et al., 2008; Molinero-Rosales et al., 1999). Moreover, constitutive expression of *ALF* does not affect the inflorescence architecture in transgenic petunia (Souer et al., 2008). In fact, in Solanaceae, the *UFO* homologs *DOUBLE TOP* (*DOT*) in petunia and *ANANTHA* (*AN*) in tomato are specifically expressed in FMs, and they are both necessary and sufficient to specify FM identity (Souer et al., 2008; Lippman et al., 2008).

In the sunflower family, Asteraceae, the inflorescence forms a pseudanthium, or "false flower". While it superficially resembles a solitary flower, the Asteraceae inflorescence is actually a tightly packed, compressed head (capitulum) composed of morphologically and functionally different types of flowers. In the sunflower, for example, showy ray flowers are formed at the capitulum periphery, while smaller disc flowers appear at the center. Individual flowers emerge in left- and right-turning spirals, the numbers of which follow the famous Fibonacci series. The entire structure is surrounded by involucral bracts that perform sepal-like, protective functions. The rapid tribal radiation of the Asteraceae family, one of the two largest among flowering plants, may correlate with this complex architecture (Bremer, 1994; Funk et al., 2009). Nonetheless, the evolutionary origin and patterning of the head-like inflorescences has been heavily debated, with some proposing that the capitulum represents a single highly-compressed raceme or cyme (Cronquist, 1977), or a condensed structure combining both cymose and racemose branching orders accounting for the evolution of floral polymorphy (Pozner et al., 2012). The latter hypothesis is based on morphological studies of the closest relatives of Asteraceae (Menyanthaceae, Goodenicaceae,

Calyceraceae) which show complex inflorescences in which the main axis shows racemose branching while the basal first-order branches follow a cymose pattern (Endress, 2010; Pozner et al., 2012). Pozner et al. (2012) proposes that a major change during the evolution of the capitulum has been the suppression of cymose patterning of these peripheral branches. Additionally, it has been proposed that the capitulum has evolved from a single, determinate and expanding meristem that through subdivision gave rise to multi-flowered head (Claßen-Bockhoff and Bull-Hereñu, 2013).

We are using *Gerbera hybrida* as a model to explore the molecular control of flower type differentiation and inflorescence development in Asteraceae. Gerbera represents the basal Mutisiae tribe within Asteraceae (Panero and Funk, 2002), and harbors heterogamous inflorescences consisting of morphologically and functionally different types of flowers. The large and showy marginal ray flowers are female, as are the smaller, intermediate trans flowers, whereas the central disc flowers are hermaphroditic and produce functional pollen. We conducted functional analyses with the two key FM identity genes *GhLFY* and *GhUFO* in gerbera to test the compelling hypotheses for the evolutionary origin of the capitulum type inflorescence. We hypothesized that if the capitulum arose from a single meristem, genetically-induced loss of FM identity would show similar phenotypic changes in both flower types. On the other hand, a condensation of racemose and cymose units could be reflected by distinct phenotypes that correlate with alterations of *Arabidopsis*-like and Solanaceae-like expression domains, respectively, for these floral regulators. Our experiments, however, reveal a novel expression pattern and function for *GhLFY*, suggesting that neither of these hypotheses alone can explain early patterning and evolution of capitulum architecture. Instead, GhLFY imparts a homeotic, FM-like identity for the entire gerbera IM.

RESULTS

GhLFY and GhUFO show specific expression patterns during capitulum development

Gerbera orthologs of the key FMI identity genes *LFY* and *UFO* were identified, cloned and characterized (Supplemental Fig. 1). Their expression was absent from vegetative tissues (leaves, floral scape or stem) and was shown to be restricted to young capitula with emerging flower primordia by quantitative RT-PCR (Supplemental Fig. 2). In addition, we found that the biochemical functions of GhLFY and GhUFO proteins are conserved *in planta* and *in vitro*. As previously found for *Arabidopsis AtLFY* (Weigel and Nilsson, 1995), ectopic expression of the orthologous gene *GhLFY* in transgenic *Arabidopsis* converted both the apical and lateral meristems into terminal flowers (Supplemental Fig. 3). Moreover, overexpression of *AtUFO* and *GhUFO*, respectively, led to formation of supernumerary petals (Lee et al., 1997; Supplemental Fig. 3). GhLFY and GhUFO proteins were also shown to physically interact with each other in yeast two-hybrid assay (Supplemental Fig. 4). As in case of AtUFO (Chae et al., 2008), LFY interaction only occurred when the F-box region was removed from GhUFO.

The expression domains of *GhLFY* and *GhUFO* were further investigated by *in situ* hybridization in gerbera. Transcripts of both genes were absent from the vegetative shoot apical meristem (SAM) as well as leaf primordia (Fig. 1A,E). In *Arabidopsis*, *AtLFY* expression is strictly localized to the determinate flower meristem (Weigel et al., 1992). However, we show that, after the reproductive transition, *GhLFY* is expressed uniformly in the naked, dome-shaped IM, an early expression domain, suggesting that the capitulum is in fact a determinate structure (Fig. 1B). *GhLFY* expression in capitula persisted throughout floral primordia development (Fig. 1B,C) and localized to the emerging involucral bract primordia, where it appeared exclusively in their adaxial domain, and later on, in the axils of each involucral bract (Fig. 1C). During flower primordia initiation, *GhLFY* expression was visible in the incipient flower primordia already before their outgrowth (Fig. 1C,G). In contrast, *GhUFO* was not expressed in the naked inflorescence meristem (Fig. 1E). Its

expression initiated temporally later, and was restricted solely to the emerging floral primordia (Fig. 1F,M). During patterning of individual flowers, both *GhLFY* and *GhUFO* show conserved expression patterns comparable to those found in *Arabidopsis* and petunia (Souer et al., 2008; Lippman et al., 2008; Ingram et al., 1995). During early stages, the expression of both *GhLFY* and *GhUFO* localizes to the central region of FMs (Fig. 1G,M), to the marginal pappus bristle (sepal) primordia (Fig. 1H,N), and to the boundaries of petal primordia (Fig. 1I,O). Later on, *GhUFO* and *GhLFY* show complementary expression domains. *GhUFO* is restricted to the bases of petals (Fig. 1P,Q), while *GhLFY* transcripts are found in all four whorls of floral organs (Fig. 1J,K).

GhLFY is required to maintain the determinacy of the capitulum inflorescence meristem

We created transgenic gerbera lines to explore the detailed functions of FMI genes in gerbera. Transformation of RNAi constructs efficiently suppressed *GhLFY* and *GhUFO* expression, respectively (Fig. 2G,H), and led to highly modified inflorescence phenotypes (Fig. 2). As expected, in the most severe transgenic lines, we observed loss of floral organ identities and conversion into green, leaf- or bract-like organs (Fig. 2C,E). We also identified milder phenotypes with only partially reduced expression of *GhLFY* and *GhUFO*, respectively and accordingly less pronounced loss of organ identities (Fig. 2D,F). Scanning electron microscopic analyses of the early stages of inflorescence patterning in the transgenic lines uncovered that GhLFY has evolved a novel and specific role in Asteraceae in defining determinacy of the IM (Fig. 3). In wild-type gerbera, the capitulum produces a fixed number of flowers until the center of the meristematic area is fully consumed with disc flower primordia (Fig. 3A,D) (Uimari et al., 2004; Teeri et al., 2006). In transgenic lines with suppressed *GhUFO* expression, IM was similarly consumed as in wild-type (Fig. 3C,F) but, in contrast, the capitula of *GhLFY* RNAi lines remained undifferentiated (Fig. 3B,E). In these plants, disc flower primordia first develop regularly from the margins toward the center as in wild type, but their emergence ceases at a certain stage whereupon they start to initiate

in a random manner in the center of the IM (Fig. 2B,E). We did not detect any signs of consumption of capitulum growth in these lines, as new primordia kept initiating constantly even when the inflorescence was fully open and most of the flowers had reached anthesis.

Ectopic expression of GhUFO confers floral fate to the capitulum

The flower primordia-specific expression of *GhUFO* suggests that it may be a master gene that defines FM identity. We produced transgenic lines with precocious, ectopic expression of *GhUFO* under the CaMV35S promoter (Supplemental Fig. 5) and found no effect on vegetative growth, while reproductive structures were highly modified (Fig. 4A,B). The emerging young capitulum was more elongated than expanded, and it retained its small size throughout the development (Fig. 4B). The involucral bracts surrounding the capitula were arranged in a spiral order as in wild-type, but they gained increasing petaloid identity toward the center of the capitulum. This phenotypic change correlated with upregulation of B class MADS box genes (Supplemental Fig. 5). However, instead of forming flower primordia in spiral phyllotaxis, the meristem showed whorled phyllotaxis and initiated floral organ primordia in concentric rings (Fig. 4C-E). Inwards of the petaloid bracts, true petals initiated from a fused, ring-like meristem (Fig. 4D-F) followed by several whorls of staminodes or stamen-like structures (Fig. 4D-F) that occupied the center of the capitulum. Scanning electron microscopy was used to verify the identities of these organs (Fig. 4F). Furthermore, RNA *in situ* hybridization indicated expected expression patterns of MADS box organ-identity genes in the modified organs (Fig. 4G). Expression of the B class gene *GGLO1* (Broholm et al., 2010) marked the petaloid involucral bracts and the petal "whorl" of the transgenic capitulum, and overlapping expression of both *GGLO1* and the C function gene *GAGA2* (Yu et al., 1999) was confined to staminodes and stamens (Fig. 3G).

In transgenic lines with milder phenotypes, the change from an inflorescence program to floral organ formation occurred temporally later, suggesting that post-transcriptional output or a response threshold for GhUFO/GhLFY determines the switch. In the weaker lines, the peripheral primordia were still patterned as single flowers, but the inner whorls formed floral organs following an order normally found in single flowers: pappus bristles (sepals, whorl 1) followed by petals (whorl 2), stamen-like structures (whorl 3) and staminoid carpels (whorl 4) (Fig. 4D,E). Our data show that ectopic expression of *GhUFO* alone is able to trigger floral fate of the meristem and to establish a whorled pattern of floral organ development. The transgenic phenotypes indicate that the meristem recapitulates the floral pre-pattern as found in single flowers of wild-type gerbera.

Ray flowers show a distinct ontogenetic pattern associated with GhLFY function

The key feature of the Asteraceae capitulum is the presence of distinct flower types. Close studies of the early ontogeny of distinct flower types in gerbera indicate that ray flower initiation deviates from those of the other flower types (Fig. 5, Supplemental Fig. 6). It is temporally delayed, and in fact, the first visible flowers are the trans flower primordia, which continue the phyllotactic pattern established by involucral bracts (Fig. 5A-C). We also show that ray flowers develop exclusively in the axils of the last series of involucral bracts (Fig. 5C). Transgenic gerbera lines with suppressed FMI gene expression formed modified capitula with leaf- or bract-like organs. However, detailed phenotypic analysis revealed unique responses in these lines. While ray flower initiation occurred similarly in wild-type and *GhUFO* RNAi lines, suppression of *GhLFY* expression showed a specific effect at early initiation stage of the ray primordia (Fig. 5D-F). The capitulum periphery in *GhLFY* RNAi plants initiated oval-shaped primordia, considerably larger than the single ray primordia of wild-type or *GhUFO* RNAi lines. These large primordia were not patterned as single flowers, but were instead further branched into two to three primordia clearly distinguished from the neighboring trans flowers (Fig. 5E). In addition, in both the wild-type and *GhUFO* RNAi lines,

213 organogenesis in ray flower primordia was delayed in comparison with trans flowers, whereas
214 patterning of the ray primordia in *GhLFY* RNAi lines occurred without any delay, again suggesting
215 an indispensable role for GhLFY in early ray flower development (Fig. 5G-I). Intriguingly, this
216 ontogenetic pattern of peripheral primordia development shares similarities with the branched,
217 peripheral cymose units found in the immediate sister family of Asteraceae, Calyceraceae (Pozner et
218 al., 2012), or the syncephalium subunit primordia (SSP) described in species of Asteraceae with
219 higher-order capitulum structures, i.e., those bearing capitula within capitula (Harris, 1994; 1999).
220 Our data suggest that ray primordia in Asteraceae may have evolved through suppression of
221 branching of the peripheral cymose units still found in Calyceraceae capitula and by gain of floral
222 fate defined by GhLFY.

223

224 **Floral patterning is regulated by GhLFY and GhUFO in a flower-type dependent manner**

225 After flower primordia initiation, at the level of single flowers, loss of FM identity in both *GhLFY*
226 and *GhUFO* RNAi lines was obvious. We conducted SEM analyses in order to characterize the
227 phenotypic changes more closely. In the most severe transgenic lines, suppression of either *GhLFY*
228 or *GhUFO* expression led to complete loss of floral organs, and emerging primordia repeatedly
229 initiated secondary (P2) and tertiary (P3) flower primordia subtended by bract-like structures (Fig.
230 6A,B). A similar strong phenotype was observed in a mutant gerbera cultivar, Pingpong, which
231 lacks *GhUFO* expression (Fig. 7A, I-J). This cultivar was identified from a breeder's collection as
232 its inflorescence resembles the transgenic *GhLFY* and *GhUFO* phenotypes (Fig. 7A). In Pingpong,
233 the marginal ray primordia initiate as in wild type and *GhUFO* RNAi lines and the capitulum is
234 fully packed with flower primordia (Fig. 7D,E). Furthermore, the single primordia continuously
235 produce secondary and tertiary primordia surrounded by bract like organs (Fig. 7F-H), leading to
236 extensive proliferation of the inflorescences (Fig. 7B,C). Altogether, our data reveal the necessary

and evolutionarily conserved roles of both GhLFY and GhUFO functions for proper patterning of individual flowers.

We also explored floral patterning in *GhUFO* and *GhLFY* RNAi lines with milder phenotypes (Supplemental Fig. 7). In these weak lines, the ray flowers responded independently from the other flower types and produced secondary flowers as in the more severe lines. In the mild *GhUFO* RNAi lines, the disc flower primordia also initiated secondary primordia, while the trans flower primordia were patterned as single flowers (Fig. 6D, Supplemental Fig. 7). The mild *GhLFY* RNAi lines instead showed an opposite effect; the disc flower primordia were patterned as single flowers, and the trans flower primordia included secondary primordia (Fig. 6C, Supplemental Fig. 7). These phenotypic changes correlated with the expression levels of the downstream B, C and E function MADS box homeotic genes, the expression of which was lacking in the most severe transgenic lines and in the mutant cultivar Pingpong, while still visible in disc flower primordia of milder *GhLFY* RNAi lines and trans flowers of *GhUFO* RNAi lines (Fig.s 6E, 7K-M). Altogether, these data suggest that both GhUFO and GhLFY form a functional gradient across the capitulum, with GhLFY showing a more pronounced role in the development of peripheral ray and trans flowers, and GhUFO in the patterning of disc flowers.

DISCUSSION

The unique feature of the Asteraceae plant family is that their inflorescences mimic solitary flowers. This homeotic transference of flower-like identity to the inflorescence was likely the key innovation for the diversification of this largest family of flowering plants. Here, using the model plant *Gerbera hybrida*, we provide functional data suggesting that the capitulum not only resembles a single flower at morphological level, but also at molecular level. Our data further demonstrates that transitions in plant reproductive evolution use conserved genetic modules and pathways that can be

261 readily co-opted for adaptive success. We show a conserved function for *GhUFO* of gerbera in
262 specifying FMI while the *LFY* homolog *GhLFY* has evolved novel functions in defining the
263 determinacy of the IM and in regulating development of the marginal ray flowers. Our data suggests
264 that distinct flower types have independent evolutionary origins.

265

266 **The capitulum is a determinate structure that confers floral fate upon ectopic *GhUFO***
267 **expression**

268 We identified the key floral meristem identity genes in gerbera in order to understand patterning of
269 the capitulum architecture and get insight into its evolutionary origin. As is common to most
270 angiosperms, the flower meristem identity genes *GhLFY* and *GhUFO* were found as single copy
271 genes in gerbera. Their functions in a heterologous *Arabidopsis* background as well as their
272 capacity to physically interact with each other indicate that their protein functions have been
273 conserved between rosids and asterids. However, we show that both spatial and temporal
274 modifications in the expression domains of these regulators have played a major role in the
275 evolution of capitulum architecture (Fig. 8A). Our data indicates that the expression pattern of
276 *GhLFY* has diversified, showing uniform, early expression in the dome-shaped inflorescence
277 meristem before initiation of flower primordia. This stands in contrast to the indeterminate
278 *Arabidopsis* raceme, where TERMINAL FLOWER1 (TFL1) activity in both the apical and lateral
279 inflorescence meristems inhibits the expression of flower meristem identity genes *LFY* and *AP1*,
280 and consequently flower initiation (Weigel et al., 1992; Bradley et al., 1997). Similarly to petunia
281 with its cymose inflorescences, the UFO ortholog of gerbera (GhUFO) defines FM identity (Figure
282 8A). In petunia, the WOX homeodomain protein EVERGREEN (EVG) specifies lateral IM (SIM)
283 development and is needed to activate *DOT* (Rebocho et al., 2008). Whether the WOX-like proteins
284 in gerbera play a role in meristem patterning remains to be studied.

The expanding gerbera IM lacks a terminal flower. Suppression of *GhLFY* expression led to loss of determinacy and random initiation of flower primordia in the centermost region of the inflorescence meristem. Moreover, ectopic expression of *GhUFO* was able convert the capitulum into a large single flower. We observed upregulation of both B and C genes in accordance with the identities of single organs. As the *GhUFO* RNAi lines showed absence of B and C gene expression (Fig. 6), we suggest that *GhUFO* is needed to regulate both these organ identity genes as also previously found in petunia (Souer et al., 2008). Altogether, *GhUFO* expression re-established the whorled floral patterning and led to development of single floral organs in the capitulum background, indicating that the gene has a conserved and indispensable role in defining FMI even when transferred to the whole-inflorescence level.

The fact that *GhLFY* has evolved a broader expression domain in Asteraceae suggests that it is involved in regulation of early inflorescence meristem growth. It has previously been proposed that apart from floral identity determination, the ancestral function of LFY was in promoting meristematic growth more generally, by affecting cell division (reviewed in Moyroud et al., 2010; Teo et al., 2014). In legumes, *LFY* mutants develop simpler leaves, indicating the involvement of LFY function in leaf indeterminacy (Hofer et al., 1997; Hofer and Ellis, 2002; Dong et al., 2005). Furthermore, in grasses LFY homologs are required to maintain indeterminacy of the early panicle meristem affecting branching architecture of the inflorescences (reviewed in Moyroud et al., 2010; Kyozuka et al., 2014). Moreover, in Arabidopsis LFY stimulates the growth of axillary meristems (Moyroud et al., 2010). Our findings indicate that in Asteraceae LFY shows further functional diversification. Uniform expression of *GhLFY* across the naked capitulum defines it as a determinate structure resembling the single floral meristems borne on racemes or cymes (Weigel et al., 1992; Souer et al., 2008). The capitulum can thus be seen as an analog of a single flower not just functionally or by its ontogeny, but also at the molecular level. Still, as-yet uncharacterized factors are contributing to compression and/or fusion of the capitulum.

310

311 **LFY has a specific role in regulating the ontogeny of ray flowers**

312 It is commonly presented that flower primordia in Asteraceae heads develop acropetally, from the
313 margins towards the center following the spiral phyllotaxis. Harris (1995) found such acropetal
314 development to be strictly limited to species that display capitula consisting of only a single flower
315 type. Species with true ray flowers, however, show a consistent delay in ray flower development.
316 Furthermore, bidirectional development of flower primordia was documented for several species
317 (Harris, 1995). For example, in *Erigeron philadelphicus*, outermost disc flowers are the first to
318 emerge, and the subsequent disc flowers initiate acropetally. The first ray flowers initiate
319 proximally next to the oldest disc flowers (from non-peripheral starting points), and the next ones
320 develop basipetally towards the involucral bracts, suggesting that ray flower development is not
321 directly derived from that of disc flowers (Harris et al., 1991). We similarly show that the early
322 ontogeny of peripheral ray flowers is different from the other flower types. In gerbera, the ray
323 primordia initiated temporally later than the closest trans flowers. We found that *GhLFY* expression
324 localized to the base of the involucral bracts already at a stage when these bracts started to initiate.
325 These cells with localized *GhLFY* expression may represent suppressed axillary structures, as
326 *GhLFY* silencing specifically converted the ray primordia into branched peripheral units.
327 Furthermore, our data indicated that the temporal delay in ray flower patterning was regulated by
328 GhLFY.

329 We also observed radial gradients for both the GhLFY and GhUFO functions, with GhLFY
330 function being more pronounced at the periphery and GhUFO in the center of the capitulum.
331 Interestingly, a similar gradient along the inflorescence axis is also found in *Arabidopsis lfy* mutant,
332 which shows stronger phenotypes in early arising meristems compared to the younger ones (Weigel
333 et al., 1992). In gerbera, this gradient was already reflected in our previous work that investigated
334 differential expression of several MADS box genes across the capitulum (Laitinen et al., 2006;

Kotilainen et al., 2000; Yu et al., 1999) as well as specific expression of CYCLOIDEA2-like TCP domain transcription factor genes in ray flower primordia (Broholm et al., 2008; Tähtiharju et al., 2012), emphasizing the key role the FMI genes play in defining flower type differentiation. However, what exactly creates this gradient and what its actual nature is remain obscure. The *Arabidopsis AtLFY* is known to be regulated by auxin (Yamaguchi et al., 2013), but still, the putative role of auxin in regulating patterning of inflorescences, either racemes or capitula, remains to be studied.

Evolution of capitulum architecture

Condensation of the elongated inflorescence into a capitulum is an example of convergent evolution as it has occurred several times independently during the evolution of Asteridae and other taxa (Harris, 1999). In this paper, our focus is on Asteraceae primary capitula, which have the most basic type of head-like inflorescences combining ray and disc flowers into a single structure. We show that in gerbera GhLFY has a specific role in defining ray flower development. Suppression of *GhLFY* expression converted the ray flower primordia into large meristems that subdivided and formed branched structures. The ontogeny of these structures was similar to the so called "cymose units" found in the Calyceraceae, a close relative of Asteraceae (Pozner et al., 2012) (Fig. 8B). We therefore propose that the differential development of gerbera ray flowers is not related to their marginal position per se but instead to their distinct ontogenetic origin as separate, cymose inflorescence axes, and correspondingly, that LFY has contributed to a gain of floral fate for the peripheral branching units found in the capitula of Calyceraceae (Pozner et al., 2012). Interestingly, the ontogeny of branched primordia in *GhLFY* RNAi lines also shares similarity with the "syncephalium subunit primordia (SSP)" that are found in secondary flower heads of several Asteraceae taxa (Harris, 1995). A syncephalium is a higher-order aggregation of capitula (capitula within capitula) that evolved more recently than the simple capitulum, and it is often accompanied

by a reduction of the primary inflorescences to a single-flowered state (Harris, 1995). Our data therefore suggest that LFY function may play a role in regulating development of secondary flower heads as well.

CONCLUSIONS

Inflorescence architecture display enormous variation in nature and their architecture play a central role in reproductive fitness and adaptation of plants, as well as crop yield. The evolutionary success of the large Asteraceae plant family has been associated with the unique head-like inflorescences consisting of different flower types. Here we show that the highly conserved flower meristem identity regulator LFY has evolved novel functions in regulating inflorescence development in Asteraceae. Unlike in other model species LFY specifies the development of the naked inflorescence meristem and specifically regulates ray flower development. Our data supports the hypothesis that flower type differentiation in Asteracae is connected to their different developmental origin.

MATERIALS AND METHODS

Plant material

Gerbera hybrida (Asteraceae) cultivars Terraregina and Pingpong (Terra Nigra BV, The Netherlands) were grown under standard greenhouse conditions as described previously (Ruokolainen et al., 2010b). The developmental stages for gerbera flower primordia development are described by Laitinen et al. (2006) and Tähtiharju et al. (2012).

Isolation of *GhLFY* and *GhUFO* cDNAs and genetic transformation of gerbera

The gerbera homologs for *LFY* and *UFO* were searched from the gerbera EST sequence database and the full length cDNAs were amplified with gene specific primers (Supplemental Table 1). Total RNA was isolated from young inflorescences (5-10 mm in size) with Trizol reagent (Invitrogen) and first strand cDNA (Boehringer first strand cDNA kit) was used as a template for PCR. The full length cDNAs were cloned into Gateway entry vector pDONR221 (Invitrogen) and were verified by sequencing. For genetic transformation, we used the Gateway binary vectors pK7WG2D and pK7GWIWG2D (II) (Karimi et al., 2002) to generate overexpression and RNAi constructs, respectively. The gene constructs were electroporated into the *Agrobacterium* strain C58C1 harboring pGV3101. Transformation of the gerbera cultivar Terraregina with *GhUFO* overexpression and *GhLFY* and *GhUFO* RNAi constructs was done according to Elomaa and Teeri (2001).

Expression analysis

Expression analyses on transgenic plants were conducted by quantitative RT-PCR. To verify the efficiency of RNA interference (Figure 2), we selected three independent *GhLFY* RNAi (TR6, TR1,

TR7) and two *GhUFO* RNAi (TR9, TR1) lines for expression analysis. Sampling was done by dissecting inflorescences of 2-3 mm in diameter for *GhLFY* RNAi plants and 4-5 mm in diameter for *GhUFO* RNAi plants together with wild type control of corresponding sizes. For the mutant cultivar Pingpong, inflorescences were 3-4 mm in diameter showing a similar developmental stage of flower primordia as the 4-5 mm Terraregina inflorescences. To verify the expression of the downstream floral homeotic genes in transgenic Terraregina lines and Pingpong, individual flower primordia at different developmental stages were sampled. For *GhLFY* and *GhUFO* RNAi plants, we dissected primary (P1) and secondary (P2) primordia from three flower types according to their morphology (Figure 6A-D) under stereomicroscopy. For comparison, we collected stage 3 primordia of three different flower types from wild type (Laitinen et al., 2006). Since there were no clear flower type specific responses in cultivar Pingpong, we collected the centermost primary and secondary primordia for expression analysis (Figure 7).

The expression analysis of 35S:*GhUFO* plants was done by semi-quantitative RT-PCR. Young leaves (3-4 mm) from 6 independent transgenic lines (TR1, TR2, TR4, TR5, TR6, TR10) were sampled for RT-PCR to verify ectopic expression of GhUFO in comparison with WT (Supplemental Figure 5). Furthermore, involucral bracts with petaloid identity were collected from fully opened inflorescences (lines TR1, TR2, TR5 and TR6) to check the expression of B class MADS box genes (*GGLO1* and *GDEF2*) that define petal identity in gerbera (Yu et al., 1999; Broholm et al., 2010) (Supplemental Figure 5).

Expression analysis of *GhLFY* and *GhUFO* RNAi lines was conducted with quantitative RT-PCR. Total RNA was extracted with the CTAB method modified from Chang et al. (1993). After precipitation, the pellet was washed in 70% ethanol and dissolved in RNase-free water. DNase treatment was performed according to the instructions of the RNA clean-up kit (Macherey-Nagel, Germany). cDNA synthesis (500 ng) and qPCR was conducted as previously (Tähtiharju et al., 2012). The qPCR primers are shown in Supplemental Table 1. For testing the *GhLFY* and *GhUFO*

RNAi efficiency, we normalized *GhLFY* and *GhUFO* expression level to the expression of gerbera *GAPDH* reference gene according to the $^{\Delta\Delta}$Ct method (Pfaffl, 2001). The expression levels of downstream MADS box genes were normalized against the total RNA amount used for cDNA synthesis as stated in Tähtiharju et al. (2012). Expression analysis was conducted for the B class MADS box genes *GGLO1* (Yu et al., 1999), *GDEF1*, *GDEF2* and *GDEF3* (Broholm et al., 2010) as well as C class genes *GAGA1* and *GAGA2* (Yu et al., 1999).We also included the *SEPALLATA*-like E class genes. In gerbera, *GRCD1* and *GRCD2* have been shown to specifically affect stamen and carpel development, respectively (Kotilainen et al., 2000; Uimari et al., 2004) while *GRCD4* and *GRCD5* provide redundant and general E function in gerbera (Ruokolainen et al., 2010a). The quantitative RT-PCR results were analyzed in three biological replicates. For construction of the heatmap, we used log- transformed average means of relative expression level.

In situ hybridization

For *in situ* expression analysis, the preparation of the plant samples, sectioning and hybridization were done as in Elomaa et al. (2003). Gene specific probes for *GhLFY* (294 bp), *GhUFO* (299 bp), and *GAGA2* (288 bp) were synthesized as in Juntheikki-Palovaara et al. (2014) using a PCR-amplified fragment of the given gene with primers containing a few extra nucleotides and a T7 overhang (taatacgactcactataGGGAGG or CAtaatacgactcactataGGG) in the 5′ end (Supplemental Table 1), and labelled following the instructions of the DIG RNA labelling Kit (Roche). The probe corresponding to *GGLO1* (217 bp) was the same as in Broholm et al. (2010). Sections were examined and photographed using the Leitz Laborlux S Microscope equipped with Leica DFC420 C Digital Camera.

Scanning Electron Microscopy

For SEM analysis of the wild type and transgenic gerberas, a time series of capitula at early developmental stages were hand dissected under stereomicroscopy. When necessary, excessive involucral bracts were removed to expose the center inflorescence meristem and the developing flower primordia. Sample preparations were performed as previously in Uimari et al. (2004) except an automated Leica EM CP300 dryer was used for critical point drying. Samples were examined using the Quanta 250 (FEI) SEM at the Electron Microscopy Unit at the Institute of Biotechnology, University of Helsinki. The obtained SEM images were further edited and pseudo-colored in Adobe Photoshop CC 2015.

Accession numbers

Sequence data from this article can be found in the GeneBank under the following accession numbers: KU554694 for *GhLFY* and KU554695 for *GhUFO*.

Supplemental Data

Supplemental Data. Supplemental materials and methods.

Supplemental Figure 1. Amino acid sequence alignments of GhUFO and GhLFY with selected orthologous genes.

Supplemental Figure 2. Expression of *GhLFY* and *GhUFO* in diverse plant parts.

Supplemental Figure 3. Ectopic expression of *GhLFY* and *GhUFO* in transgenic *Arabidopsis*.

Supplemental Figure 4. Protein-protein interactions of GhLFY and GhUFO.

Supplemental Figure 5. Expression analysis and organ identities of 35S:*GhUFO* lines.

Supplemental Figure 6. Early ontogeny of gerbera capitulum development.

Supplemental Figure 7. Individual flowers in wild type and *GhLFY* and *GhUFO* RNAi lines with mild phenotypes.

Supplemental Table 1. Primer sequences used in this study.

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480 **Figure legends**

481 **Figure 1. Expression domains of *GhLFY* and *GhUFO* in the inflorescence meristem (IM) and**
482 **flower meristem (FM) of wild-type gerbera.**

483 **(A)** Expression of *GhLFY* is absent from the vegetative shoot apical meristem (SAM) and leaves.

484 **(B)** *GhLFY* shows uniform expression in the young, naked, dome-shaped inflorescence meristem
485 after reproductive transition.

486 **(C)** *GhLFY* expression marks the incipient flower primordia before their outgrowth (arrow).

487 **(D)** *GhLFY* first localizes to the adaxial side of incipient bract primordia and later to the axil (arrow)
488 of the elongated involucral bract .

489 **(E)** Expression of *GhUFO* is lacking from the inflorescence meristem.

490 **(F)** *GhUFO* expression correlates with flower meristem initiation but occurs temporally later than
491 *GhLFY* (C).

492 **(G-K)** Expression domain of *GhLFY* during early floral developmental stages.

493 **(M-Q)** Expression domain of *GhUFO* during early floral developmental stages.

494 **(L, R)** Negative controls were hybridized with the sense probes for *GhLFY* (L) and *GhUFO* (R).

495 Scale bars 50 μm.

496 AS = antisense, SE = sense, IM = inflorescence meristem, FM = flower meristem, SAM = shoot
497 apical meristem, iB = involucral bract, ad = adaxial, Pa = pappus (sepals of individual flowers), Pe
498 = petal, St = stamen, Ca = carpel.

499

500 **Figure 2. Phenotypes and expression analysis of wild type gerbera and transgenic lines with**
501 **suppressed flower meristem identity gene expression.**

502 **(A)** Mature inflorescence of non-transgenic gerbera.

503 **(B)** Non-transgenic gerbera from the abaxial side. The inflorescence is surrounded by green
504 involucral bracts.

(C) Phenotype of a strong transgenic *GhLFY* RNAi line.

(D) Phenotype of a mild transgenic *GhLFY* RNAi line.

(E) Phenotype of a strong transgenic *GhUFO* RNAi line.

(F) Phenotype of a mild transgenic *GhUFO* RNAi line.

(G) Expression analysis of three independent transgenic lines with suppressed *GhLFY* expression compared to wild type (WT) gerbera.

(H) Expression analysis of two independent transgenic lines with suppressed *GhUFO* expression compared to wild type (WT) gerbera.

Scale bars 1 cm (A-F).

Figure 3. Inflorescence meristem phenotypes in transgenic lines with suppressed flower meristem identity gene expression.

(A) Wild type inflorescence meristem (IM). The center of the expanding IM has not yet been consumed by emerging flower primordia.

(B) The IM in *GhLFY* RNAi line shows random initiation of flower primordia.

(C) The IM in *GhUFO* RNAi line develops similarly as in wild type (A).

(D) Later stage of wild type IM. The IM is fully consumed with disc flower primordia.

(E) The IM in *GhLFY* RNAi line gets never consumed with flower primordia.

(F) Later stage of IM in *GhUFO* RNAi line. The IM is similarly consumed with flower primordia as in wild type (D).

Scale bars 500 µm.

Figure 4. Constitutive expression of *GhUFO* confers a floral fate to the capitulum.

(A) General phenotype of the transgenic 35S:*GhUFO* inflorescence.

(B) The young capitulum is elongating in 35S:*GhUFO* rather than expanding as in wild type (WT).

(C) Ectopic *GhUFO* leads to highly modified floral structures with organ primordia emerging in a whorled phyllotaxis. In this line with a strong phenotype, instead of single flowers, floral organs are initiated from the margins toward the center of the capitulum.

(D,E) A mild phenotype (D) and close-up of the mild phenotype (E) showing that normal flower primordia (FP) are first initiated at the capitulum periphery. The fused ring-like meristem produces petals (Pe) surrounded by pappus bristels (Pa). The innermost whorls are occupied by staminode-like organs (Std) and a mixture of stamen-like structures (St) and staminoid carpels (St/Ca).

(F) Epidermal cell structures of floral organs in WT and 35S:*GhUFO* plants. In 35S:*GhUFO*, the petaloid involucral bracts show mixtures of petal (Pe) and bract (iB) like cell types; the fused ring-like meristem show petal identity, the sterile staminodes are as in WT ray flowers and functional stamens as in WT disc flowers. We also found variants showing staminoid carpels in the center.

(G) Expression of *GGLO1* (B function MADS box gene) and *GAGA2* (C function MADS box gene) in wild type FM and 35S:*GhUFO* meristem. B gene expression is confined to petaloid bracts (iB) and petals (Pe), while both B and C genes are expressed in the staminode/stamen-like organs (St).

Scale bars 1 cm (A-B), 100 µm (C-F), 50 µm (G).

FP = flower primordia, Pa = pappus, Pe = petal, Std = staminode, St = stamen, Ca = carpel, iB = involucral bract.

Figure 5. Early ontogeny of ray primordia initiation in wild type and transgenic gerbera with suppressed flower meristem identity gene functions.

(A-C) Three consecutive developmental stages of early capitulum development in wild type gerbera. Trans flowers initiate temporally earlier than ray primordia (arrow) that emerge in the axils of last series of involucral bracts (green asterisks). **(D-F)** Scanning electron microscopy images show that the ray flower initials (shaded in yellow) of *GhLFY* RNAi plants (E) are distinct from the solitary ray primordia (shaded in red) found in wild-type (D) and *GhUFO* RNAi (F) plants.

(G-I) In contrast to wild type (G) and *GhUFO* RNAi (I) plants, the marginal ray flower primordia in *GhLFY* RNAi (H) plants show faster organogenesis compared to nearby trans flower primordia.
Scale bars 50 µm (A-C), 500 µm (D-I).

Figure 6. Patterning of the individual flower primordia in the transgenic *GhLFY* and *GhUFO* RNAi lines.

(A-B) Transgenic *GhLFY* RNAi (A) and *GhUFO* RNAi (B) plants with severe phenotypes form primary primordia (P1, shaded in yellow) that repeatedly initiate secondary (P2) and tertiary (P3) primordia (shaded in orange) in all flower types (ray, trans, disc).
(C-D) Patterning of flower primordia in *GhLFY* RNAi (C) and *GhUFO* RNAi (D) transgenic plants with milder phenotypes show flower-type specific responses. In both lines, the ray flower primordia uniformly initiate secondary primordia (P2) and consequently secondary flowers. The response in trans and disc flowers shows opposite effects; in *GhLFY* RNAi lines the disc primordia, and in *GhUFO* RNAi lines the trans primordia pattern as normal flowers (shaded in red).
(E) Heat map of quantitative RT-PCR results show expression profiles of the B, C and E function MADS box genes in developing primary (R1, T1, D1) and secondary (R2, T2, D2) primordia in different flower types.
Scale bars 100 µm (A-D).
S = severe phenotype, M = mild phenotype, P1 = primary primordia, P2 = secondary primordia, P3 = tertiary primordia, R = ray flower primordia, T = trans flower primordia, D = disc flower primordia.

Figure 7. Phenotypes and expression analysis of a gerbera mutant cultivar Pingpong.
(A-C) The inflorescence of Pingpong shows similarity with the transgenic *GhLFY* and *GhUFO* RNAi lines (A). Extensive proliferation of the inflorescences (B,C) cause splitting of the head.

(D) Patterning of the capitulum of cultivar Pingpong. The marginal primordia develop as in wild type gerbera.

(E) The inflorescence of Pingpong is fully consumed by emerging flower primordia at later developmental stage.

(F-H) Patterning of the single primordia in Pingpong. The single primordium (F) produce bract like structures surrounding the secondary primordia (G) that further initiate tertiary primordia (H).

(I) Relative expression levels of *GhUFO* in young inflorescences of wild type (WT) and the cultivar Pingpong (PP).

(J) Relative expression levels of *GhLFY* in young inflorescences of wild type (WT) and the cultivar Pingpong (PP).

(K-M) Relative expression levels of B class (K), C class (L) and SEP-like (M) MADS box genes in primary (P-1) and secondary (P-2) primordia dissected from cultivar Pingpong in comparison with the wild type stage 3 flower primordia (WT). Error bars indicate standard deviation calculated from three biological replicates (I-M).

Scale bars 1 cm (A-C) and 100 μm (D-H).

WT = wild type, PP = Pingpong, P-1 = primary primordia of Pingpong, P-2 = secondary primordia of Pingpong.

Figure 8. Functional diversification of LFY during capitulum development.

(A) In *Arabidopsis* racemes, TFL1 activity regulates the indeterminacy of the inflorescence meristem (IM) while LFY is defining flower meristem (FM) identity. In petunia cymes, the IM terminates in a flower. FM identity is defined by DOT, and the growth of the inflorescence continues from a sympodial inflorescence meristem (SIM) defined by EVG activity. In gerbera, *GhLFY* expression is uniform in the determinate IM that subdivides into single flower primordia where FM identity is defined by *GhUFO*.

605 **(B)** Suggested evolutionary pathway for capitulum development. Species representing Calyceraceae,
606 a close relative of Asteraceae, typically show branched cymose units (marked with asterisk) in the
607 periphery of their inflorescences (Pozner et al., 2012). LFY has evolved a specific role to suppress
608 branching in the marginal ray flower primordia of Asteraceae as evidenced by the *GhLFY* RNAi
609 lines.
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611

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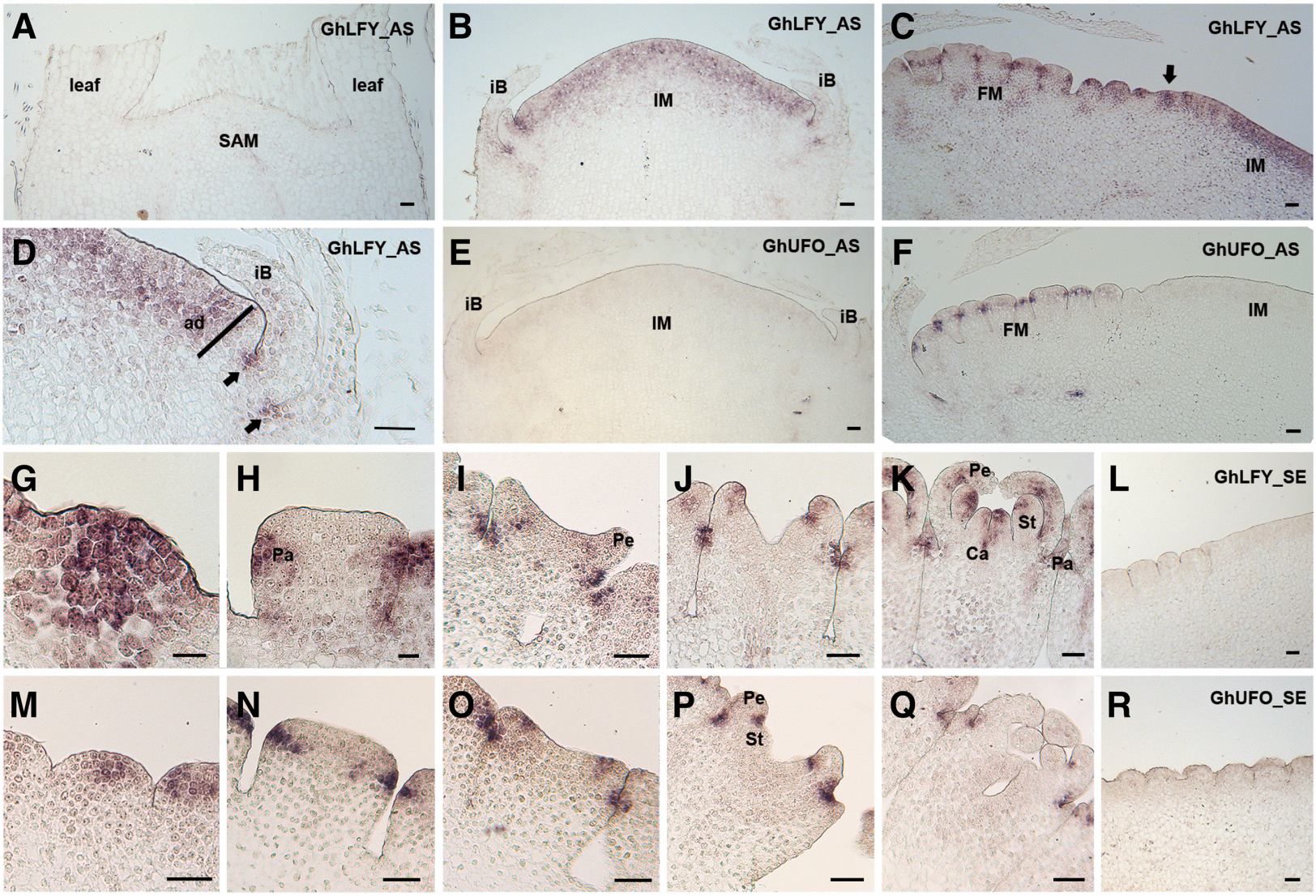

Figure 1. Expression domains of *GhLFY* and *GhUFO* in the inflorescence meristem (IM) and flower meristem (FM) of wild-type gerbera.

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(C) *GhLFY* expression marks the incipient flower primordia before their outgrowth (arrow).

(D) *GhLFY* first localizes to the adaxial side of incipient bract primordia and later to the axil (arrow) of the elongated involucral bract .

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(G-K) Expression domain of *GhLFY* during early floral developmental stages.

(M-Q) Expression domain of *GhUFO* during early floral developmental stages.

(L, R) Negative controls were hybridized with the sense probes for *GhLFY* (L) and *GhUFO* (R).

Scale bars 50 µm.

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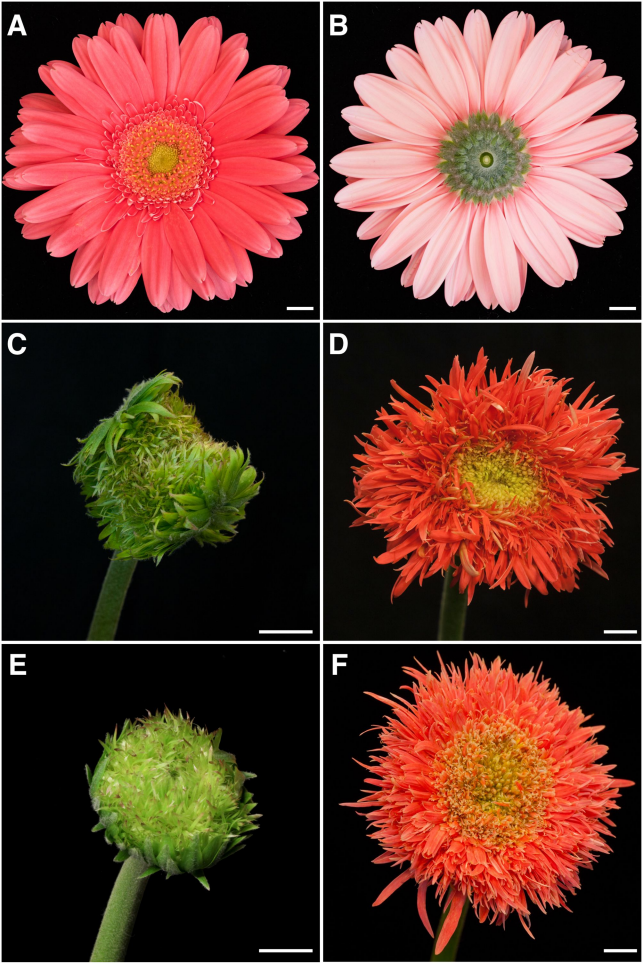

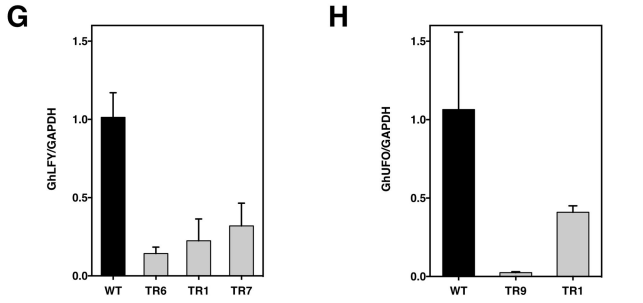

Figure 2. Phenotypes and expression analysis of wild type gerbera and transgenic lines with suppressed flower meristem identity gene expression.

(A) Mature inflorescence of non-transgenic gerbera.

(B) Non-transgenic gerbera from the abaxial side. The inflorescence is surrounded by green involucral bracts.

(C) Phenotype of a strong transgenic *GhLFY* RNAi line.

(D) Phenotype of a mild transgenic *GhLFY* RNAi line.

(E) Phenotype of a strong transgenic *GhUFO* RNAi line.

(F) Phenotype of a mild transgenic *GhUFO* RNAi line.

(G) Expression analysis of three independent transgenic lines with suppressed *GhLFY* expression compared to wild type (WT) gerbera.

(H) Expression analysis of two independent transgenic lines with suppressed *GhUFO* expression compared to wild type (WT) gerbera.

Scale bars 1 cm (A-F).

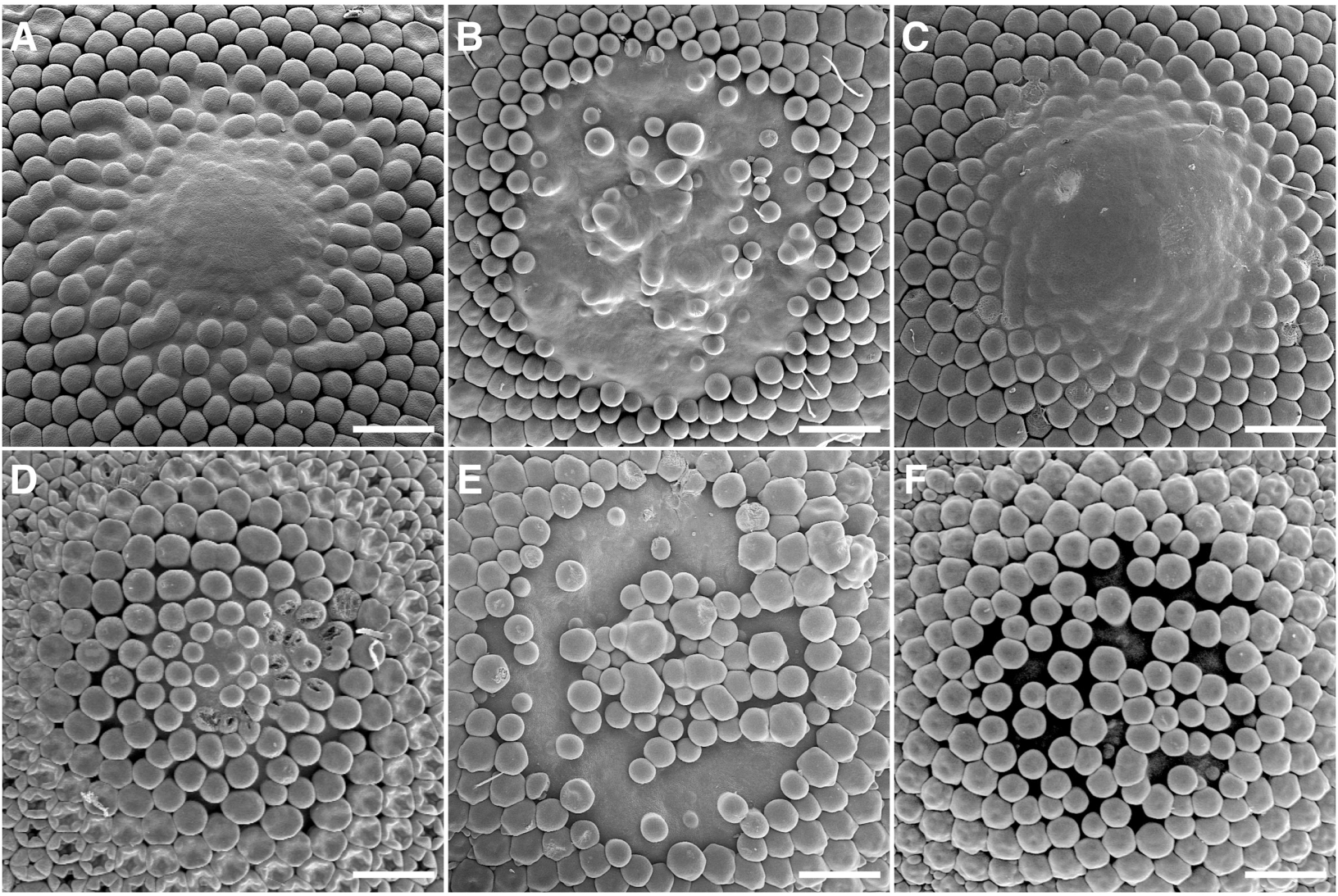

Figure 3. Inflorescence meristem phenotypes in transgenic lines with suppressed flower meristem identity gene expression.
(A) Wild type inflorescence meristem (IM). The center of the expanding IM has not yet been consumed by emerging flower primordia.
(B) The IM in *GhLFY* RNAi line shows random initiation of flower primordia.
(C) The IM in *GhUFO* RNAi line develops similarly as in wild type (A).
(D) Later stage of wild type IM. The IM is fully consumed with disc flower primordia.
(E) The IM in *GhLFY* RNAi line gets never consumed with flower primordia.
(F) Later stage f IM in *GhUFO* RNAi line. The IM is similarly consumed with flower primordia as in wild type (D).
Scale bars 500 μm.

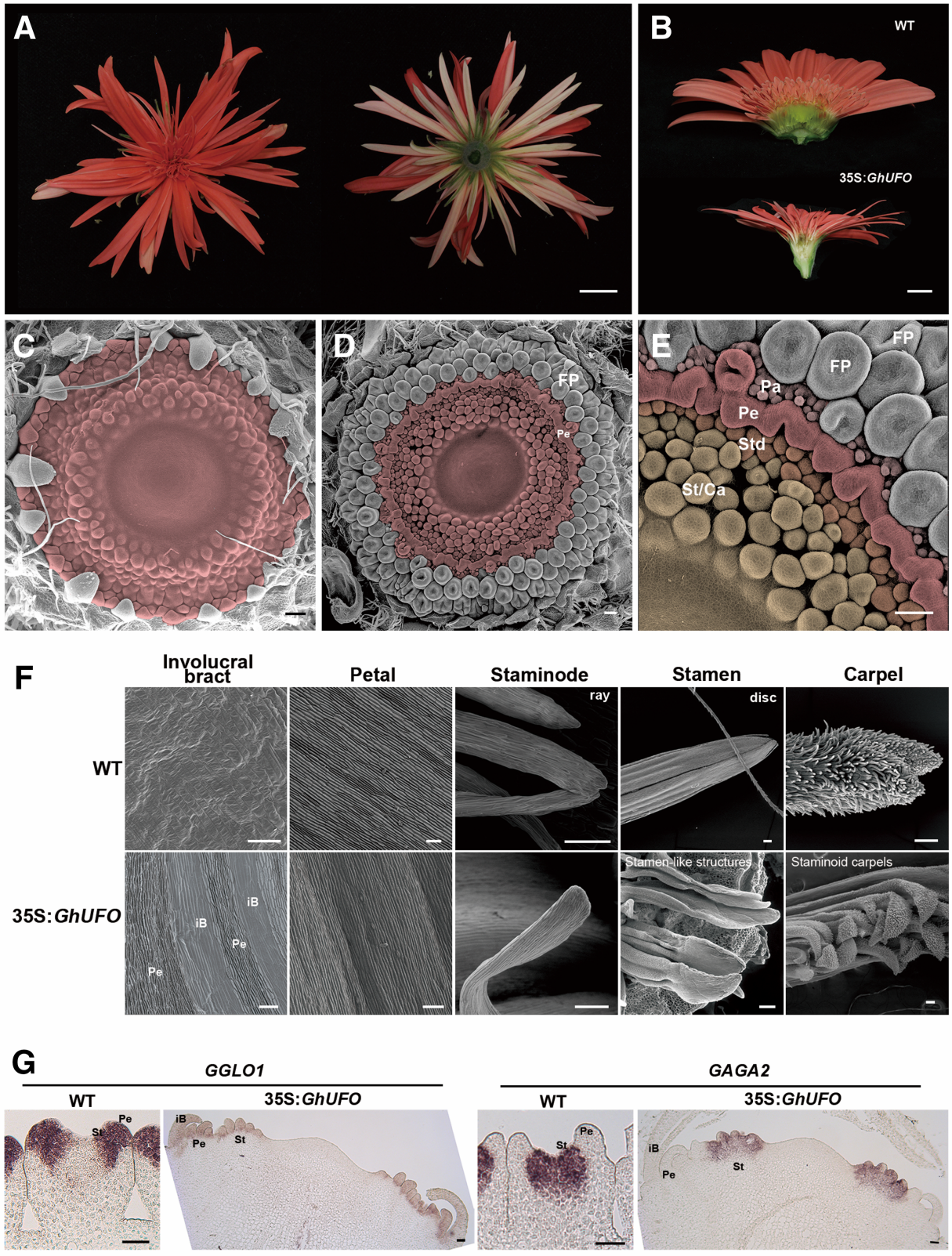

Figure 4. Constitutive expression of *GhUFO* confers a floral fate to the capitulum.
(A) General phenotype of the transgenic 35S:*GhUFO* inflorescence.
(B) The young capitulum is elongating in 35S:*GhUFO* rather than expanding as in wild type (WT).
(C) Ectopic *GhUFO* leads to highly modified floral structures with organ primordia emerging in a whorled phyllotaxis. In this line with a strong phenotype, instead of single flowers, floral organs are initiated from the margins toward the center of the capitulum.
(D,E) A mild phenotype (D) and close-up of the mild phenotype (E) showing that normal flower primordia (FP) are first initiated at the capitulum periphery. The fused ring-like meristem produces petals (Pe) surrounded by pappus bristels (Pa). The innermost whorls are occupied by staminode-like organs (Std) and a mixture of stamen-like structures (St) and staminoid carpels (St/Ca).
(F) Epidermal cell structures of floral organs in WT and 35S:*GhUFO* plants. In 35S:*GhUFO*, the petaloid involucral bracts show mixtures of petal (Pe) and bract (iB) like cell types; the fused ring-like meristem show petal identity, the sterile staminodes are as in WT ray flowers and functional stamens as in WT disc flowers. We also found variants showing staminoid carpels in the center.
(G) Expression of *GGLO1* (B function MADS box gene) and *GAGA2* (C function MADS box gene) in wild type FM and 35S:*GhUFO* meristem. B gene expression is confined to petaloid bracts (iB) and petals (Pe), while both B and C genes are expressed in the staminode/stamen-like organs (St).
Scale bars 1 cm (A-B), 100 µm (C-F), 50 µm (G).
FP = flower primordia, Pa = pappus, Pe = petal, Std = staminode, St = stamen, Ca = carpel, iB = involucral bract.

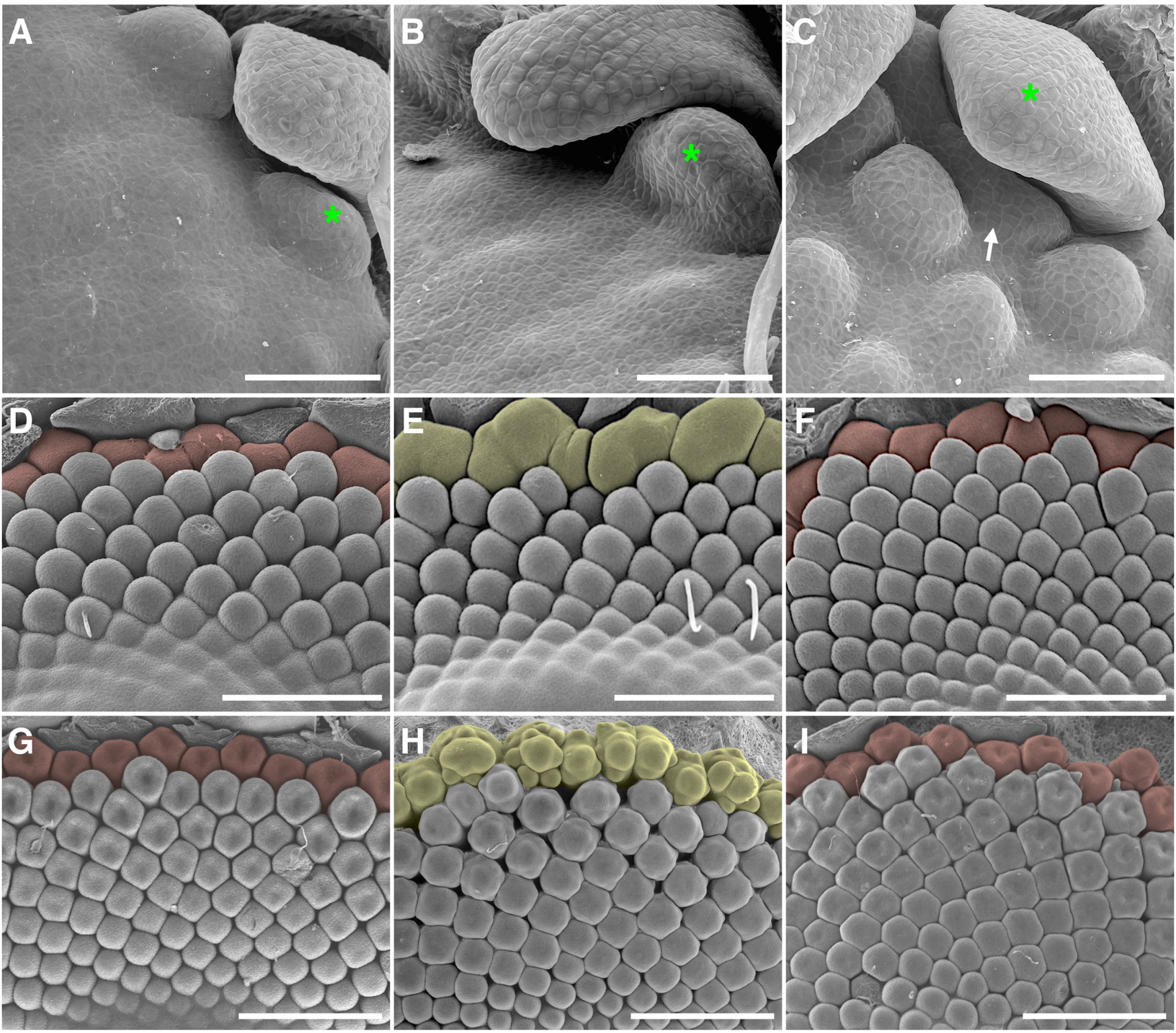

Figure 5. Early ontogeny of ray primordia initiation in wild type and transgenic gerbera with suppressed flower meristem identity gene functions.

(A-C) Three consecutive developmental stages of early capitulum development in wild type gerbera. Trans flowers initiate temporally earlier than ray primordia (arrow) that emerge in the axils of last series of involucral bracts (green asterisks).

(D-F) Scanning electron microscopy images show that the ray flower initials (shaded in yellow) of *GhLFY* RNAi plants (E) are distinct from the solitary ray primordia (shaded in red) found in wild-type (D) and *GhUFO* RNAi (F) plants.

(G-I) In contrast to wild type (G) and *GhUFO* RNAi (I) plants, the marginal ray flower primordia in *GhLFY* RNAi (H) plants show faster organogenesis compared to nearby trans flower primordia.

Scale bars 50 µm (A-C), 500 µm (D-I).

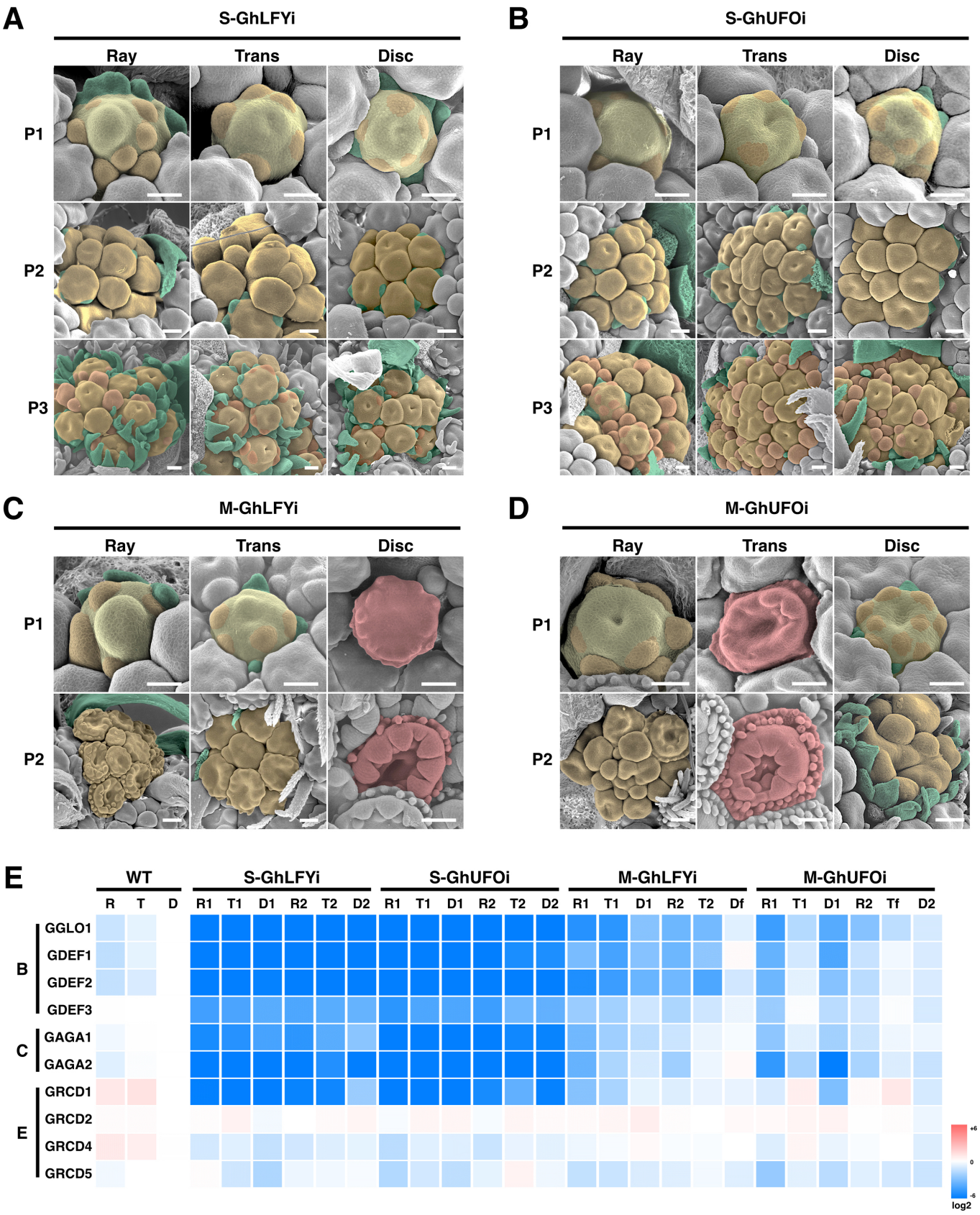

Figure 6. Patterning of the individual flower primordia in the transgenic *GhLFY* and *GhUFO* RNAi lines.
(A-B) Transgenic *GhLFY* RNAi (A) and *GhUFO* RNAi (B) plants with severe phenotypes form primary primordia (P1, shaded in yellow) that repeatedly initiate secondary (P2) and tertiary (P3) primordia (shaded in orange) in all flower types (ray, trans, disc).
(C-D) Patterning of flower primordia in *GhLFY* RNAi (C) and *GhUFO* RNAi (D) transgenic plants with milder phenotypes show flower-type specific responses. In both lines, the ray flower primordia uniformly initiate secondary primordia (P2) and consequently secondary flowers. The response in trans and disc flowers shows opposite effects; in *GhLFY* RNAi lines the disc primordia, and in *GhUFO* RNAi lines the trans primordia pattern as normal flowers (shaded in red).
(E) Heat map of quantitative RT-PCR results show expression profiles of the B, C and E function MADS box genes in developing primary (R1, T1, D1) and secondary (R2, T2, D2) primordia in different flower types.
Scale bars 100 µm (A-D).
S = severe phenotype, M = mild phenotype, P1 = primary primordia, P2 = secondary primordia, P3 = tertiary primordia, R = ray flower primordia, T = trans flower primordia, D = disc flower primordia.

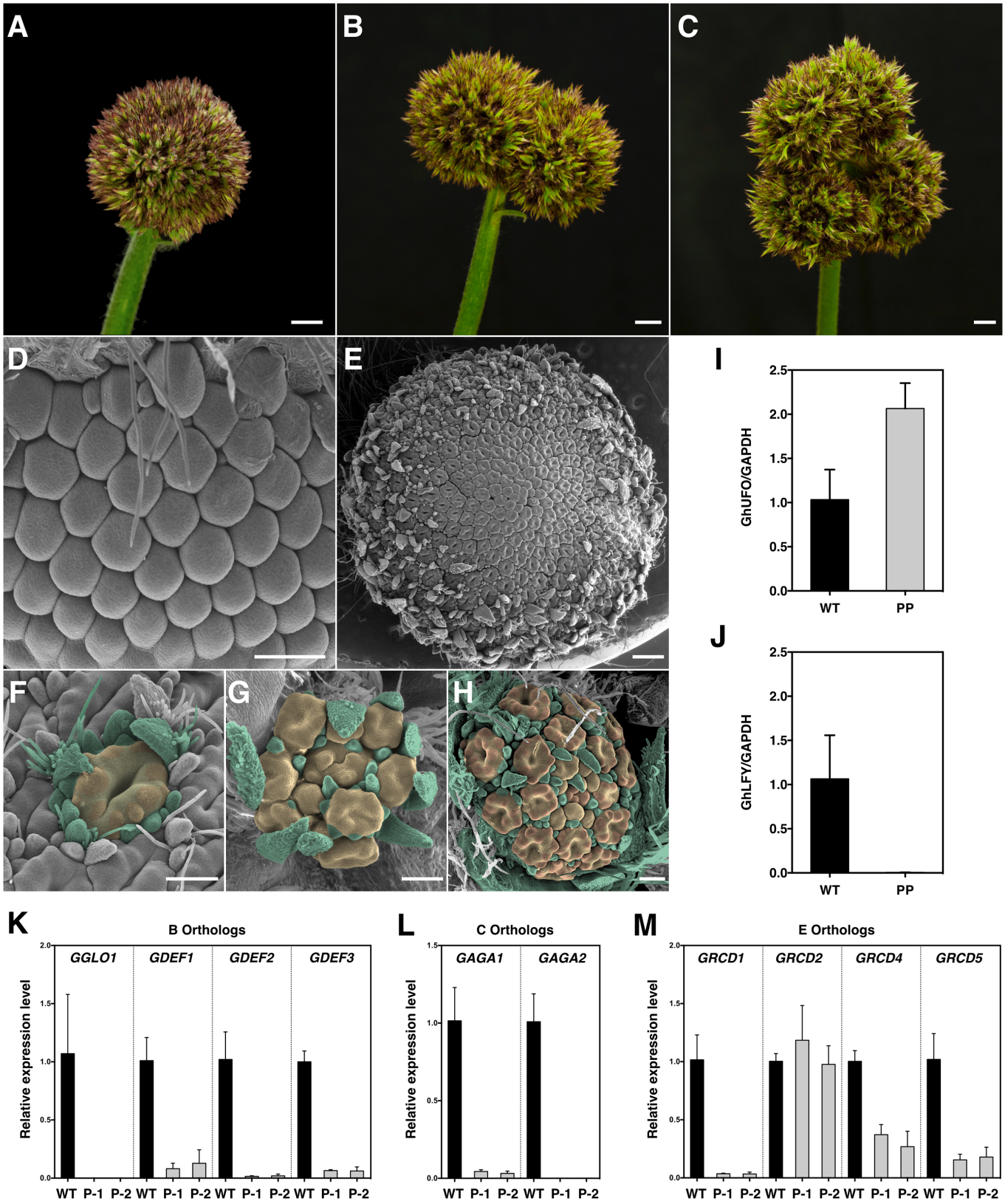

Figure 7. Phenotypes and expression analysis of a gerbera mutant cultivar Pingpong.
(A-C) The inflorescence of Pingpong shows similarity with the transgenic *GhLFY* and *GhUFO* RNAi lines (A). Extensive proliferation of the inflorescences (B,C) cause splitting of the head.
(D) Patterning of the capitulum of cultivar Pingpong. The marginal primordia develop as in wild type gerbera.
(E) The inflorescence of Pingpong is fully consumed by emerging flower primordia at later developmental stage.
(F-H) Patterning of the single primordia in Pingpong. The single primordium (F) produce bract like structures surrounding the secondary primordia (G) that further initiate tertiary primordia (H).
(I) Relative expression levels of *GhUFO* in young inflorescences of wild type (WT) and the cultivar Pingpong (PP).
(J) Relative expression levels of *GhLFY* in young inflorescences of wild type (WT) and the cultivar Pingpong (PP).
(K-M) Relative expression levels of B class (K), C class (L) and SEP-like (M) MADS box genes in primary (P-1) and secondary (P-2) primordia dissected from cultivar Pingpong in comparison with the wild type stage 3 flower primordia (WT). Error bars indicate standard deviation calculated from three biological replicates (I-M).
Scale bars 1 cm (A-C) and 100 µm (D-H).
WT = wild type, PP = Pingpong, P-1 = primary primordia of Pingpong, P-2 = secondary primordia of Pingpong.

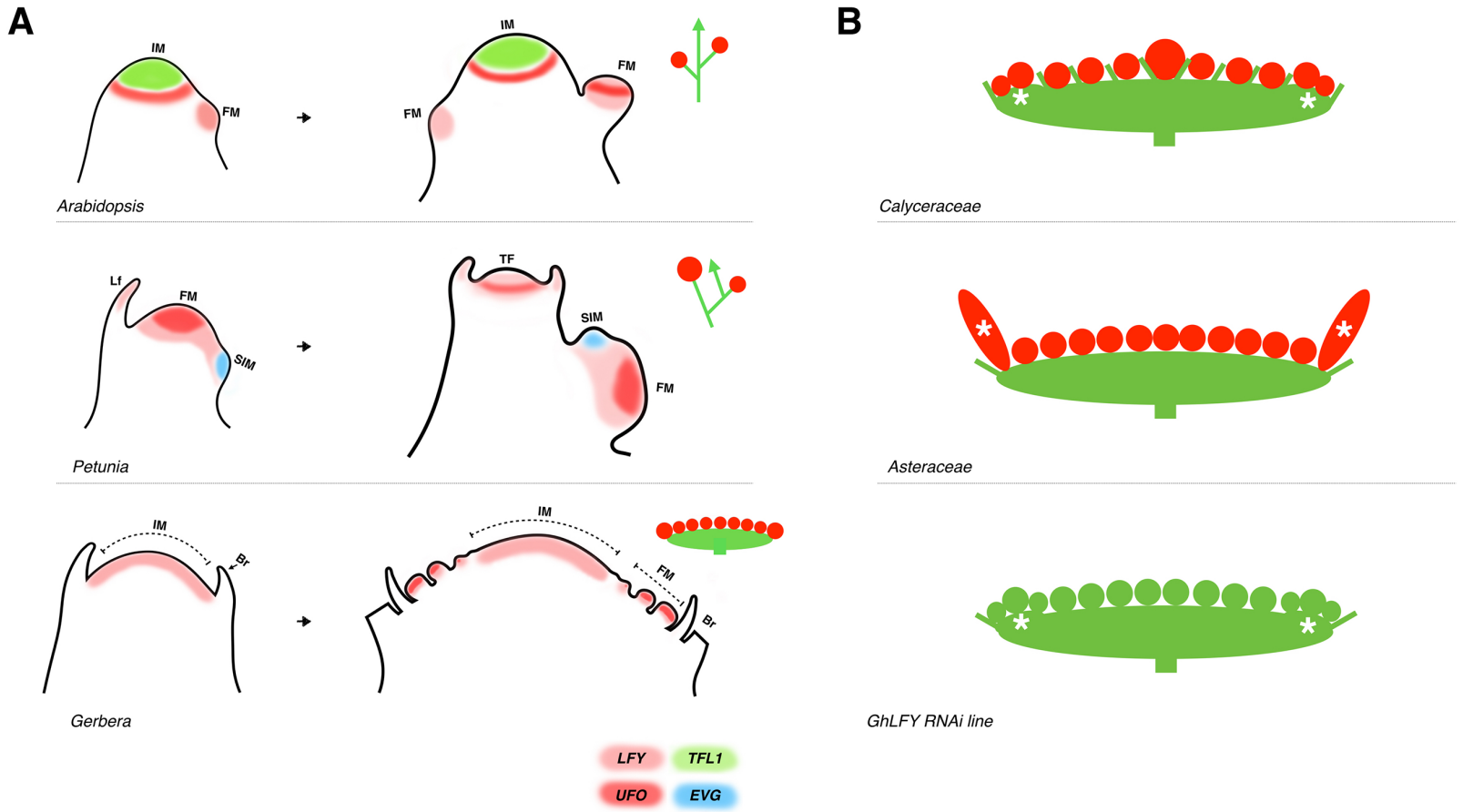

Figure 8. Functional diversification of LFY during capitulum development.
(A) In *Arabidopsis* racemes, TFL1 activity regulates the indeterminacy of the inflorescence meristem (IM) while LFY is defining flower meristem (FM) identity. In petunia cymes, the IM terminates in a flower. FM identity is defined by DOT, and the growth of the inflorescence continues from a sympodial inflorescence meristem (SIM) defined by EVG activity. In gerbera, *GhLFY* expression is uniform in the determinate IM that subdivides into single flower primordia where FM identity is defined by *GhUFO*. **(B)** Suggested evolutionary pathway for capitulum development. Species representing Calyceraceae, a close relative of Asteraceae, typically show branched cymose units (marked with asterisk) in the periphery of their inflorescences (Pozner et al., 2012). LFY has evolved a specific role to suppress branching in the marginal ray flower primordia of Asteraceae as evidenced by the GhLFY RNAi lines.

Parsed Citations